069P α_{1A}- ADRENOCEPTOR ISOFORMS DISPLAY DIFFERENT SIGNALLING PROFILES FOLLOWING AGONIST STIMULATION

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The α_1-adrenoceptor (α_1-AR) family consists of the α_{1A}, α_{1B} and α_{1D} subtypes. The α_{1A}-AR has been reported to have twelve isoforms, four of which are functional, the others being truncated and non-functional (Chang *et al* 1998). The functional isoforms denoted $\alpha_{1A\text{-}1,2,3\&4}$ have similar pharmacological properties and all couple to G_q and signal through the IP_3/DAG pathway (Chang *et al* 1998). Studies with various tissues and recombinant receptors suggest that α_{1A}-AR couple to multiple second messenger pathways including those modulated by PLA_2, PLD, adenylate cyclase, MAP kinase and protein kinase C (Graham *et al* 1996). The current study examined the ability of the four α_{1A}-AR isoforms to activate signalling pathways studied by cytosensor microphysiometry, cAMP accumulation and [^{3}H]arachidonic acid release.

Agonist stimulation of α_{1A}-AR in the cytosensor microphysiometer increased cellular activity, a summation of all the signalling pathways activated by agonist stimulation. All isoforms produced an increase in the extracellular acidification rate (ECAR) to agonists in a concentration-dependent manner. However responses of the $\alpha_{1A\text{-}2}$ to A61603 were significantly greater ($p<0.05$) than all other isoforms (E_{max} value $\mu v\ sec^{-1}$ at 80.8±8.7(4) for $\alpha_{1A\text{-}2}$, 27.9±1.9 (6) for $\alpha_{1A\text{-}1,}$ 59.7±5.0 (5) for $\alpha_{1A\text{-}3}$ and 33.0±3.0 (4) for $\alpha_{1A\text{-}4}$.) The maximal responses ($\mu v\ sec^{-1}$) of the α_{1A}-AR isoforms to noradrenaline ($\alpha_{1A\text{-}1}$-AR: 28.5±2.4 vs 40.5±2.7, (6) $P<0.0001$), methoxamine (19.4±1 vs 34.5±2.6, (6) $P<0.0001$) and A61603 (27.4±1.8 vs 36.7±2.7, (4) $P<0.0001$) were increased following pertussis toxin (PTX, 100ng/ml, 16 hr) treatment. There was no significant effect on the response to the oxymetazoline ($P=0.15$). Stimulation of cAMP accumulation occurred for the $\alpha_{1A\text{-}1,3\&4}$ isoforms in a ligand and concentration dependent manner (E_{max} % forskolin 10^{-4}M, pEC_{50}(n): A61603; $\alpha_{1A\text{-}1}$ 51.4±3.1, 8.0±0.3(4); $\alpha_{1A\text{-}3}$ 41.4±3.7, 8.8±5.3 (3); $\alpha_{1A\text{-}4}$ 38.6±2.1, 7.5±0.1(4)). Oxymetazoline had no significant effect on cAMP accumulation for any of the isoforms ($p<0.05$). The $\alpha_{1A\text{-}2}$ isoform did not produce cAMP accumulation at levels of expression comparable with the other isoforms. cAMP production was PTX sensitive for only the $\alpha_{1A\text{-}3}$-AR with an increase in the maximal response to agonist in the presence of PTX (E_{max} % forskolin 10^{-4}M: noradrenaline 31.2±4.3 vs 65.09±8.4 (4) ($P<0.0001$), methoxamine 23.64±3.7 vs 43.3±6.07 (4) ($P<0.0001$)). Stimulation of arachidonic acid release was also shown to occur for the $\alpha_{1A\text{-}1,3\&4}$ isoforms in a concentration-dependent manner with rank orders of agonist potency of cirazoline>noradrenaline>phenylephrine>oxymetazoline for $\alpha_{1A\text{-}1}$ and $\alpha_{1A\text{-}4}$ and oxymetazoline≥noradrenaline>cirazoline>phenylephrine for $\alpha_{1A\text{-}2}$-AR. The $\alpha_{1A\text{-}2}$-AR was unable to stimulate this signalling pathway at levels of expression comparable with the other isoforms (B_{max} fmol mg^{-1} protein:$\alpha_{1A\text{-}1}$:509±93(5), $\alpha_{1A\text{-}2}$: 237±66(5), $\alpha_{1A\text{-}3}$: 432±74(3), $\alpha_{1A\text{-}4}$: 433±77(5)).

These results confirm that α_{1A}-AR isoforms couple to a number of different signalling pathways, but suggest that these responses are ligand and isoform dependent.

Chang DJ *et al*., (1998) FEBS Lett. 422: 279-283
Graham RM *et al.,* (1996) Circ Res. 78: 737-49